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The BRCA1 gene was cloned in 1994. Dr. Narod participated in this international effort. Dr.Narod has been active in characterizing the range and frequency of mutations in families with hereditary breast cancer, in Ashkenazi Jewish women with familial and sporadic breast and ovarian cancer, and in unselected Ontario women with ovarian cancer. By studying over 400 BRCA1 carriers identified through the course of these studies he has identified genetic and non-genetic risk modifiers and he has helped to evaluate the outcomes of genetic counselling of hereditary breast cancer patients. He has recently found that the use of oral contraceptives is protective against hereditary ovarian cancer and a history of moderate smoking is associated with a decreased risk of breast cancer in BRCA1 carriers.

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5. Introduction

The BRCA1 gene was mapped to chromosome 17q in 1990 by Mary-Claire King and colleagues. Shortly thereafter, Dr. Narod was able to confirm that BRCA1 was the gene for the breast-ovarian cancer syndrome as well. From 1990-1994, a series of experiments in several laboratories confirmed that BRCA1 was the gene responsible for the majority of families with multiple cases of breast and ovarian cancer. The risk of breast cancer for women who carry a mutant copy of this gene is 87% to age 70. The risk of ovarian cancer is 50%. The purpose of the present study is to clone the BRCA1 gene and to identify the range of mutations present in the families with the breast ovarian cancer syndrome.

The objectives of the study were stated as follows:

1) To identify highly informative polymorphic markers in the region of BRCA1.

2) To type linked breast and ovarian cancer families with these markers and to identify all of the crossovers which provide information about the minimum region of chromosome 17 containing BRCA1.

3) To produce a physical map of the minimum region by identifying a series of overlapping contiguous fragments of YAC DNA inserts that span the region.

4) To identify the coding sequences in the cloned YACs and to assay the expression of these genes in normal breast and ovary and in tumors derived from these tissues.

5) To test genes identified to be in the minimum region for rearrangements and for point mutations that may be associated with cancer predisposition in the families.

6) To sequence the BRCA1 gene in our panel of 100 hereditary breast and breast-ovarian cancer families and to identify the range of mutations in this gene.

7) To evaluate the association between particular mutations and cancer patterns in the panel of families.

8) To sequence the BRCA1 gene in the constitutional DNA of panel of women with apparently sporadic breast and ovarian cancers.

9) To sequence the BRCA1 gene in the tumour DNA of panel of women with apparently sporadic breast and ovarian cancers.

Objectives 1 to 5 were accomplished by Dr. Narod and other researchers. These objectives were completed in 1994 and the bulk of the grant was devoted to objectives 6 - 9.

Objectives 6 and 7 were reached in 1996. These results indicated that there is little variation in phenotype by BRCA1 mutation. There is a modest decrease in ovarian cancer risk with truncating mutations in the 3' end of the BRCA1 gene. However, the risk of breast cancer appears to be the same for all BRCA1 mutations. It appears that the other genes and non-genetic factors will be more important than BRCA1 genotype in determining the lifetime risk of cancer.

Objective 8 has now been completed for 307 unselected Ontario women with ovarian cancer, 220 Jewish breast cancer families, and for 218 unselected Jewish women with ovarian cancer and for 97 French-Canadian breast cancer families.

Objective 9 has been studied by others. It is found that mutations in BRCA1 are not frequent in sporadic breast and ovarian tumours. The great majority of mutations detected have been germline (hereditary) mutations. This objective will not be pursued further.

Details of the progress of these individual objectives is provided below:

6. Body

6.0 Overview

The goal of the current study was to identify the BRCA1 gene through positional cloning. The approach involved a combination of genetic mapping and physical mapping, leading to the identification of candidate genes from chromosome 17q. The BRCA1 gene was cloned in October 1994 (Miki et al, 1994). Dr. Narod contributed to this effort by fine mapping of the gene.

The cloning of the BRCA1 gene took place in year one of the grant period of Dr Narod. Dr. Narod has capitalized on the early results and has gone on to use these results to investigate several aspects of the population genetics of BRCA1, including: establishing the range of BRCA1 mutations in breast-ovary families; 2) establishing mutation/haplotype correlations; 3) establishing the existence of predominant mutations in ethnic subgroups; 4) establishing the proportion of breast cancer families due to BRCA1; 5) establishing genetic and non-genetic modifiers of BRCA1 penetrance.

This body of work has contributed to the completion of 27 papers which are listed in the References below. Dr. Narod is the first author on four papers, is the senior author on eight other papers and is a contributing author on the 15 remaining papers.

6.1 Genetic mapping of BRCA1

In 1994 the region of assignment was established to be an interval of 1.2 kb surrounding the gene for estradiol dehydrognease (EDH17B). This was a key candidate gene and had been tentatively ruled out by direct sequence analysis, but not by linkage analysis. Dr. Narod hired a post-doctoral fellow, Dr. Patricia Tonin, to direct an effort to perform fine mapping of the BRCA1 gene by linkage analysis. Families for study originated from the cancer genetics clinic at the Montreal General Hospital and were also provided by Dr. Henry Lynch at Creighton University, Omaha. A total of 40 families from Canada and the US were studied for linkage. A panel of 16 polymorphic chromosome 17q markers was used in this analysis, spanning a region of 20cM. By examining a genetic recombinant in a 45-year old woman in a family with 10 cases of early-onset breast cancer and a single case of ovarian cancer it was possible to map the BRCA1 gene distal to the EDH17B locus (Tonin et al, 1994). This identified the proximal boundary for BRCA1. This information was shared with investigators from the University of Utah, who had recombination information that established a distal boundary for the BRCA1 interval at the locus D17S78. These two markers formed the genetic interval of assignment of BRCA1, a region of 600kb.

6.2 Physical mapping of BRCA1

At the same time as the construction of the genetic map, a physical map of cloned DNA fragments (YACS) was under construction in the laboratory of Dr Narod. In his laboratory Dr. Tonin established that three overlapping YAC fragments, HSD1, HSD2 and HSD3 contained most of the region of the 600kb of interest established by the mapping experiments. Dr. Narod then enlisted two other Canadian scientists to assist in the identification of candidate genes in this region. Dr. J. Rommens of the Hospital for Sick Children in Toronto was able to identify 30 unique cDNA fragments, by hybidisation of RNA libraries to immobilized YACs (Rommens et al, 1995). These 30 clones were aligned to form ten transcription units. These gene fragments were sequenced in the laboratory of Dr. Jacques Simard, of Quebec City. Several known and unknown genes were mapped by this process, including gamma- tubulin and the Ki antigen gene (Rommens et al, 1995). None of these 30 clones was the BRCA1 gene.

6.3 Identification of BRCA1 mutations

The information from the physical map constructed by Dr. Narod led to the mapping of several genes in the region of chromosome 17q21 but none of these were the BRCA1 gene. At the same time a similar effort took place in the laboratories of Myriad Genetics, under the direction of Mark Skolnick. Dr. Skolnick successfully identified BRCA1 in late 1994. Because of the genetic mapping information that was shared by

Dr Narod's group and Dr. Skolnick's group, Drs. Narod and Tonin were authors on the manuscript that described the cloning of BRCA1 in September 1994 (Miki et al, 1994).

After BRCA1 was identified, Dr. Narod studied families with hereditary breast and ovarian cancer for the presence of mutations. From 1994 to 1996, Dr. Narod directed four studies of mutation analysis of families with hereditary breast cancer and with the breast ovarian cancer syndrome, and has also contributed to several consortium analyses. Much of the sequencing was done in the laboratory of Dr. Simard in Quebec City as per a collaborative agreement between Dr. Narod and Simard. In the first study, 30 Canadian families with breast and ovarian cancer were studied by full length sequencing. These families had evidence of linkage to chromosome 17q. 14 BRCA1 mutations were identified (Simard et al, 1994). This experiment was noteworthy in that the first examples of recurrent BRCA1 mutations were identified in the Canadian families. In four families the mutation 185 delAG was found. This is now the most commonly reported BRCA1 mutation. In another four families a second mutation, 5382 ins C, was found. Dr. Narod generated genetic haplotypes for all gene carriers in these families. By inspection of the haplotypes, it was evident that the recurrent mutations were seen in the context of a common haplotype. (table 1) This implies that the patients with these mutations, although previously have been believed to be unrelated, have a common ancestor. A second set of Canadian families was screened for mutations. In this set of 30 breast cancer families an additional four mutations were found (Durocher et al, 1996).

Table 1 - BRCA1 mutations and corresponding haplotypes

Family	Mutation	Haplotype S855	S1322	S1323	S1327
101	**5382insC**	**D**	**E**	**F**	**0**
102	--	C	E	F	M
113	--	B/E	D/E	F	M
130	--	C	E	F	M
121	--	F/H	D	C	G/H
136	--	F/G	C/D	F/G	B/I
161	--	F	C/E	D/F	B/M
162	**5382insC**	**D**	**E**	**F**	**O/M**
164	--	D/G	B/F	C/E	E/G
166	**5382insC**	**D/G**	**C/E**	**C/F**	**F/O**
172	--	C/D	D/E	E/F	M
178	--	E	C	F	J
179	--	F/G	D/E	B/F	G/M
180	**185delAG**	**G/H**	**C/E**	**C/F**	**E/L**
183	**418del4**	**A**	**F**	**F**	**M**
185	**1293del40**	**F**	**D**	**F**	**M**
186	--	G	D	C	C
211	--	E	C	C	E
213	--	D	E	E	M
218	**3121delA**	**E**	**E**	**F**	**M**
235	**185delAG**	**G**	**C**	**C**	**E**
240	--	E	E	F	M
247	--	F	D/E	F	M
248	--	A/G	D	C/F	D/M
253	**185delAG**	**G/H**	**C/D**	**B/C**	**E/F**
254	--	D/H	D	C/F	M/C
255	**185delAG**	**G**	**C**	**C**	**E**
259	--	C/F	E/G	E/F	M
270	**1128insA**	**G**	**D**	**B**	**D/I**
279	**5382insC**	**D**	**E**	**F**	**O**

-- No mutation identified.

A further 50 breast-ovarian cancer families from Creighton University were characterized by a combination of linkage analysis, haplotype analysis and direct sequencing. The families originated from the cancer family database of Dr. Henry Lynch of Creighton University. The linkage analysis and haplotype analysis was done in the laboratory of Dr. Narod. The sequencing was done in the laboratory of Dr. Gilbert Lenoir in Lyon, France. In the first report, there were 16 BRCA1 mutations identified in the 20 families from Creighton University (Serova et al, 1996). A second set of 30 families from Creighton University have been characterized for BRCA1 and BRCA2 mutations (Serova et al, 1997). Thirteen mutations were identified.

The linkage and mutation data that is generated for the Creighton families is used for a genetic counselling evaluation project that is also funded by the Department of the Army (Caryn Lerman PI). Dr. Narod provided risk assessment data used for this study based on linkage analysis and direct sequencing of mutations and has to date provided DNA-based risk assessments to over 250 individuals in these 20 families. The funds for the laboratory component of this study are derived from the current project *The Cloning of the BRCA1 Gene.* Dr Narod does not receive any additional funding for this project from Dr. Lerman. Several factors were found to predict utilization of genetic testing in this study, including sex, educational status and insurance coverage (Lerman et al, 1996, Lerman et al, 1997).

6.4 Genetic Epidemiology of BRCA1

After the cloning of BRCA1 much of the effort in Dr. Narod's laboratory focused on studies of the range and frequency of BRCA1 mutations in the population and the use of this information for the rapid detection of mutations.

In the first paper of BRCA1 mutations in Canada it was noted that there were four families reported with the 185 delAG mutation from the 13 families with mutations (Simard et al, 1994). It was noted that all of these were of Ashkenazi Jewish origin. Two additional Canadian families with this mutation were discovered. Overall, the two base pair deletion was seen in 6 of 24 families with BRCA1 mutations. All six families of these families were Jewish, compared to 1 of 18 families with other BRCA1 mutations ($p < 0.0001$). The report of Tonin et al (1995) was the first description of the association of Jewish ancestry and specific BRCA1 mutations. It was then found by another research group that this mutation was present in 1% of Ashkenazi Jews in North America. In an attempt to establish the importance of founding mutations in Jewish breast ovarian cancer families, Dr. Narod established a collaborative research group, involving nine centers from North America. A total of 220 Jewish families with two or more cases of breast cancer were studied. Mutations were found for 100 of 220 Jewish families with two or more cases of breast cancer (Tonin et al, 1996). Mutations were found in 25 of 28 families with two or more cases of breast cancer and two or more cases of ovarian cancer. Dr. Narod was the leader of this collaboration and did

the statistical analysis for the group and wrote the manuscript. Tonin was the post-doctoral fellow of Dr. Narod at the time of this study and was first author. (table 2)

Table 2. Frequency of BRCA1 and BRCA2 Mutations in Jewish Breast Cancer Families

		MUTATION (%)				
	Total Families	**185delAG**	**5382insC**	**6174delT**	**Any**	**(%)**
Site Specific Breast Cancer (no ovarian cancer)						
2 breast cancers	48	10	2	0	12	(25.0%)
3 breast cancers	43	7	3	1	11	(25.6%)
4+ breast cancers	47	11	2	4	17	(36.1%)
Total	138	28	7	5	40	(29.0%)
Breast-Ovarian Cancer Syndrome						
2+ breast, 1 ovarian	54	22	9	4	35	(64.8%)
2+ breast, 2+ ovarian	28	21	4	0	25	(89.3%)
Total	82	43	13	4	60	(73.2%)

One of the critical questions is to estimate the proportion of Jewish women with breast and ovarian cancer who are carriers of the delAG mutation. To answer this question Dr. Narod directed a study of 218 Jewish unselected patients with ovarian cancer. These patients were identified from the list of all living patients with epithelial ovarian cancer at each of 12 hospitals in Canada, Israel and the USA. Data was collected by Dr. Narod and his research team from each of the hospitals. Ms Roxana Moslehi was a graduate student of Dr. Narod assigned to this project. She interviewed each patient about her family history and her reproductive history and a blood sample was obtained. The DNA was extracted in the laboratory of Dr. Narod and screened for the 185 delAG and the 5382 insC mutations and the 6174 delT mutation of BRCA2. Of the 218 women

enrolled in this study there have been 80 mutations detected (36.7%) including 41 women with 185delAG mutations, 15 with the 5382insC mutations and 24 with the 6174 delT BRCA2 mutation (Moslehi et al, in preparation). The age of diagnosis of the women with BRCA2 mutations was significantly older than the age of the women with BRCA1 mutations.

Dr. Narod completed BRCA1 mutation analysis on a panel of 309 unselected incident cases of ovarian cancer diagnosed in the province of Ontario. The specimens were collected through a grant to Dr. Harvey Risch of Yale University. The mutation analysis was entirely performed in the laboratory of Dr. Narod under the direct supervision of his laboratory supervisor, Dr John Abrahamson. Dr. Abrahamson in a molecular biologist and is currently supervising the mutation detection efforts in the laboratory of Dr. Narod. (Risch et al, submitted). 307 women with incident ovarian cancer were screened by the protein truncation test, and using specific ASO assays for common mutations. Twenty six mutations were identified. The frequency of mutations in 8.5% of women was much higher than previous reports. Mutations were present in 14% of cases of invasive serous carcinoma. No mutations were found in cases of borderline or cancers (table 3).

Table 3. Prevalence of mutations by type of ovarian cancer

Type	Total	Mutations	Percent
Serous	215	24	11.1%
Mucinous	44	1	2.3%
Endometrioid	41	1	2.4%
Borderline	46	0	0.0%

Mutations in BRCA1 were more likely in women with young onset ovarian cancer; however BRCA2 was more important for ovarian cancers occurring after age 60 (table 4).

Table 4. Prevalence of BRCA1 and BRCA2 mutations by age of onset of ovarian cancer.

Age group	BRCA1 carriers n = 16	BRCA2 carriers n = 10	No mutation n = 283
mean age	50.3 years	60.4 years	56.2 years
<40	5.4%	0%	94.6%
41-50	12.7%	1.6%	85.7%
51-60	5.5%	4.4%	90%
61+	0.8%	4.2%	95.0%

Dr. Narod analysed and prepared a report of data from 145 breast-ovarian cancer families on behalf of the Breast Cancer Linkage Consortium (Narod et al, 1995a). The study estimated that 76% of breast ovary cancer families were attributable to BRCA1. If the family had no cases of male breast cancer and two or more cases of ovarian cancer, the estimated linked proportion was 92%. After the BRCA1 gene was cloned and BRCA2 was mapped it was possible to reanalyse the data from the 145 families (Narod et al, 1995b). There were 10 apparently unlinked families in the original report. BRCA1 mutations were subsequently identified in three families and the other seven families are linked to BRCA2. None of the original 145 families is convincingly unlinked to BRCA1 and to BRCA2. Dr. Narod contributed to a second consortium report which estimated the proportion of breast cancer families linked to both genes and the penetrance of breast and ovarian cancer in carriers of both genes (Ford et al, 1998). Dr. Narod has made the largest contribution to the Breast Cancer Linkage Consortium of the North American collaborators.

Not all carriers of BRCA1 mutations develop cancer, and for those who do, the age of onset varies. Some women develop breast cancer and other develop ovarian cancer. In the first attempt to determine the relevant non-genetic factors which contribute to the clinical expression of the BRCA1 gene, Dr. Narod collected clinical information from 333 female carriers of BRCA1 mutations (Narod et al, 1995c). By using of a historical cohort design, and a Cox proportional hazards analysis, it was been possible to study the effect of reproductive factors on cancer penetrance. It was found that parity is an important risk modifier - each additional birth decreased the risk of breast cancer by

15%. There was a strong cohort effect present for both cancer types; the risk of breast and ovarian cancer is roughly double for women born after 1930 than for women born before 1930.

In 1996, Dr. Narod provided the first evidence that the risk of cancer in BRCA1 carriers could be modified by the alleles at other genetic loci. Dr. Narod and his graduate student, Catherine Phelan, studied the influence of rare alleles of the HRAS1 polymorphism on breast and ovarian cancer risk in BRCA1 carriers. Dr. Phelan developed an assay for the detection of rare alleles of the HRAS gene based on PCR amplification followed by Southern blotting. The alleles were sized by electrophoresis on an agarose gel. The frequencies of alleles of different sizes in the patient populations was established. A rare allele was defined as one whose frequency was less than 2%. Ms. Phelan genotyped 307 individuals with BRCA1 mutations for whom DNA samples were available in the laboratory of Dr. Narod. Overall, a rare allele was present in 17 of the 42 women with ovarian cancer, compared to 51 of the 265 women without ovarian cancer ($p < 0.05$). The presence of a rare allele of the HRAS1 polymorphism was associated with a 2.85-fold increase in the risk of ovarian cancer in the cohort of 307 BRCA1 carries ($p = 0.002$). There was no effect on the penetrance of breast cancer by the HRAS1 locus (Phelan et al, 1996).

Two recently completed studies have shown strong modifying effects of oral contraceptives and smoking on cancer risk in carriers of BRCA1 and BRCA2 mutations.

Dr. Narod performed a case-control study of 207 cases of ovarian cancer among BRCA1 and BRCA2 carriers. Much of the mutation data was generated from the laboratory of Dr. Narod. In addition, data were submitted to Dr. Narod from 13 collaborating centers in eight countries. Overall this study revealed a 60% reduction of ovarian cancer risk associated with six or more years of oral contraceptive use (Narod et al, submitted).(table 5)

Table 5 Association between Oral Contraceptive Use and Ovarian Cancer Risk

	All controls included		BRCA1/2 carrier controls only
OC variable	**Univariate analysis** odds ratio (95% CI)	**Multivariate analysis** odds ratio (95% CI)	**Multivariate analysis** odds ratio (95% CI)
Ever-never	0.44 (0.29-0.68)	0.53 (0.33-0.85)	0.35 (0.17-0.74)
OC duration			
Trend, per year of use	0.91 (0.87-0.95)	0.92 (0.87-0.96)	0.91 (0.85-0.98)
OC duration (years)			
never used	1.00	1.00	1.00
0-3 years	0.67 (0.38-1.16)	0.79 (0.44-1.42)	0.40 (0.17-0.94)
3-6 years	0.36 (0.18-0.72)	0.44 (0.22-0.90)	0.35 (0.12-1.01)
≥6 years	0.33 (0.19-0.57)	0.39 (0.22-0.70)	0.30 (0.12-0.73)

Footnote to table 5. Multivariate odds ratios are adjusted for year of birth, parity, and country of residence. Multivariate analyses of BRCA1/2 carriers additionally include mutation type (BRCA1 or BRCA2). Each OC variable is considered in a separate model. The right hand column includes only 53 control sisters who are confirmed mutation carriers.

The greatest known modifier of breast cancer risk in BRCA1 and BRCA2 carriers appears to be cigarette smoking. Dr. Narod conducted a matched case-control study of breast cancer among 186 breast cancer cases and 186 matched controls. Both cases and controls had BRCA1 or BRCA2 mutations. Active smokers were found to be at roughly one-half the risk of breast cancer compared to non-smokers (Brunet et al, in press; Mr. Brunet is the statistician employed by Dr. Narod).

Table 6. Smoking histories in BRCA1 carriers with and without breast cancer

Variable	Breast Cancer	Controls	p-value
pack years	4.51	6.14	0.01
packs per week	1.88	2.77	0.02

In a multivariate analysis, smoking greater than four pack-years was associated with an odds ratio of 0.46 for developing breast cancer among BRCA1/BRCA2 carriers ($p = 0.006$).

7. CONCLUSIONS

Through a linkage approach Dr. Narod defined the smallest interval containing the BRCA1 in 1994. This information enabled Skolnick and colleagues to clone this gene in 1994. The identification of this gene has been followed by several studies of Dr. Narod regarding the frequencies of BRCA1 mutations in women with familial and non-familial breast and ovarian cancer. Dr. Narod identified a recurrent Jewish mutation 185 del AG which accounts for 20% of unselected Jewish women with ovarian cancer. Dr. Narod's efforts have also focused on establishing the presence of genetic and non-genetic modifiers of cancer risk in BRCA1 carriers. The HRAS gene is the first gene found to modify the risk of ovarian cancer in BRCA1 carriers. The most important non-genetic factors which modify the risk of breast and ovarian cancer in BRCA1 and BRCA2 carriers are smoking and oral contraceptive pills.

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9.0 SALARIED PERSONNEL

1. Adrienne Scott
2. Jean-Sebastien Brunet
3. Marie Claud Facuher
4. Andrew Manning
5. Karen Robb
6. Amy Paulson
7. Rafik Rahgeb
8. Zhou Lishen
9. John Abrahamson